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Background and Aims: Tamoxifen (Tam) is the most commonly used drug to treat estrogen receptor (ER)-positive breast cancers (BC). This drug is an estradiol antagonist, which has previously been demonstrated to restrain growth and trigger oxidative stress and cell death of ER-positive breast cancer cells. In spite of the high effectiveness of endocrine therapy, onset of drug resistance accounts for both tumor recurrence and reduced survival of BC patients. Previous studies showed that autophagy is dysregulated in Tam-resistant BC cell lines and tumors, supporting the view that autophagy may critically contribute to drug resistance.
Aim of this research is to gather further information on the putative mechanisms underlying Tam-induced cell death and to better delineate the role of autophagy dysregulation in anticancer drug resistance in BC cells.
Methods: Human ER-positive MCF7 cells were treated with Tam, after which viability (MTT assay) and clonogenic capability were measured. Integrity of the lysosomal compartment was assayed by staining the cells with either acridine orange or LysoTracker Red for live-cell microscopy, in basal conditions and following transient overexpression of metallothionein 2A (MT2A).
Results: The results show that 20 μM Tam induces intense cellular vacuolization and cell death within hours. The acidic compartment is heavily affected already after 2 h of treatment: lysosomes lose their capability of either take up or to retain dyes such as acridine orange or LysoTracker Red, indicating that the drug elicits lysosomal membrane permeabilization (LMP). Tam also markedly reduces clonogenic survival of MCF7 cells; this effect is significantly abrogated by pretreatment with 100 μM vitamin E and, to a lower extent, with the iron chelator deferiprone. The finding that also LMP is effectively attenuated by vitamin E strongly supports the possibility that oxidative stress and intracellular iron play a key role in Tam-induced LMP and death. We have thus investigated whether overexpression of MT2A affected in some way Tam-induced LMP. MT2A-overexpressing MCF7 cells evidenced a lysosomal localization of overexpressed MT2A and displayed a significantly reduced LMP compared to control cells. These data strongly support the view that dysregulation of iron-chelating proteins, as well as of their subcellular localization, markedly modulate cellular susceptibility to Tam-induced LMP.
Conclusions: These preliminary results indicate that, at the concentrations used, Tam rapidly triggers LMP and cell death in MCF7 cells. These non-canonical and still poorly characterized biological effects seem to rely on oxidative stress and might prove to be important for anticancer activity of the drug. The results also show that Tam-induced LMP is effectively counteracted by overexpression of iron-chelating proteins, thus suggesting that the dysregulation of iron-binding proteins may contribute to increase the resistance of BC cells to anticancer drugs.